



Frequency of *Mycobacterium tuberculosis*-specific CD8⁺ T-cells in the course of anti-tuberculosis treatment



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ARTICLE INFO

Article history:

Received 1 December 2014

Received in revised form 16 January 2015

Accepted 16 January 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:

CD8⁺ T-cells

Mycobacterium tuberculosis

Tetramers

Multimers

MHC

SUMMARY

Anti-tuberculosis drug treatment is known to affect the number, phenotype, and effector functionality of antigen-specific T-cells. In order to objectively gauge *Mycobacterium tuberculosis* (MTB)-specific CD8⁺ T-cells at the single-cell level, we developed soluble major histocompatibility complex (MHC) class I multimers/peptide multimers, which allow analysis of antigen-specific T-cells without ex vivo manipulation or functional tests. We constructed 38 MHC class I multimers covering some of the most frequent MHC class I alleles (HLA-A*02:01, A*24:02, A*30:01, A*30:02, A*68:01, B*58:01, and C*07:01) pertinent to a South African or Zambian population, and presenting the following MTB-derived peptides: the early expressed secreted antigens TB10.4 (Rv0288), Ag85B (Rv1886c), and ESAT-6 (Rv3875), as well as intracellular enzymes, i.e., glycosyltransferase 1 (Rv2957), glycosyltransferase 2 (Rv2958c), and cyclopropane fatty acid synthase (Rv0447c). Anti-TB treatment appeared to impact on the frequency of multimer-positive CD8⁺ T-cells, with a general decrease after 6 months of therapy. Also, a reduction in the total central memory CD8⁺ T-cell frequencies, as well as the antigen-specific compartment in CD45RA–CCR7⁺ T-cells was observed. We discuss our findings on the basis of differential dynamics of MTB-specific T-cell frequencies, impact of MTB antigen load on T-cell phenotype, and antigen-specific T-cell responses in tuberculosis.

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1. Introduction

Cell-mediated immune responses are essential for protection against tuberculosis (TB).¹ CD8⁺ T-cells produce cytotoxic molecules as well as cytokines. Cytotoxic T-cells are capable of killing cells infected with *Mycobacterium tuberculosis* (MTB),² while aiding the recruitment and activation of other immune cell types. CD8⁺ T-cells recognize short antigen-derived peptides (epitopes) presented on major histocompatibility complex (MHC) class I molecules on the surface of antigen-presenting cells (APCs).³ Clinical and preclinical assessment of CD8⁺ T-cells in TB show that

CD8⁺ T-cells play an important role in recall responses, as well as in long-term protection to MTB infection.^{2,4}

Only a limited number of T-cell epitopes of 9–11 amino acids in length have been identified to date,⁵ using technologies such as ELISA,⁶ ELISPOT,⁷ the chromium release assay,⁸ the thymidine incorporation assay,⁹ intracellular cytokine staining (ICS),¹⁰ and soluble MHC class I/peptide multimers.¹¹ A large number of these epitopes originate from only a few well-characterized, immunologically relevant MTB proteins, e.g., early-secreted antigenic target 6 kDa (ESAT-6, Rv3875),¹² culture filtrate protein 10 kDa (CFP10, Rv3874),⁷ antigen 85B (Ag85B, Rv1886c),¹¹ TB10.4 (Rv0288),¹³ and a conserved transmembrane protein (Rv1733c).¹⁴ This represents a rather biased view of T-cell responses to only a handful of over 4000 existing MTB proteins potentially expressed during infection.¹⁵ From another 60 MTB proteins, one or only several T-cell epitopes

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have been identified – leaving the majority of the MTB proteome to be explored for T-cell responses.

Since the expression of MTB proteins is associated with different stages of MTB growth as well as TB disease,¹⁶ the identification of a broader MTB epitope profile is essential for the discovery of markers associated with active or latent TB infection (LTBI). Half of the MTB epitopes associate with a single MHC class I allele (HLA-A*02:01) out of more than 3000 different alleles described in humans thus far.¹⁷ Based on this information, it is clear that more CD8+ T-cell epitopes derived from novel MTB antigens associating with clinically relevant MHC class I alleles need to be described, especially since the regional distribution of alleles varies between continents as well as different ethnic groups.¹⁸

One technique to identify epitope-specific (MTB antigen-specific) T-cells without in vitro manipulation is the use of MHC class I multimers. We have previously designed a broad panel of MHC class I multimers covering seven different MHC alleles presenting epitopes from six different MTB-derived proteins:¹⁹ the early expressed secreted antigens TB10.4 (Rv0288), Ag85B (Rv1886c), and ESAT-6 (Rv3875), as well as intracellular enzymes glycosyltransferase 1 (Rv2957), glycosyltransferase 2 (Rv2958c), and cyclopropane fatty acid synthase (CFA synthase; Rv0447c). Since anti-TB drug treatment is known to affect the number, phenotype, and effector functionality of antigen-specific CD8+ T-cells,^{20,21} we chose to develop and work with MHC class I multimers in this study as a means to decipher the nature of antigen-specific CD8+ T-cells before and after anti-TB drug therapy.

2. Materials and methods

2.1. Patient data

Seven HIV patients with untreated, active pulmonary TB, diagnosed with at least two positive sputum smears for acid-fast bacilli or positive sputum culture for MTB, were enrolled at the University of Stellenbosch, South Africa, as well as from the University Hospital Lusaka, Zambia. Five were female and two were male; they ranged in age from 20 to 55 years. Samples were taken at the time of diagnosis (before initiating treatment) and after 6 months of treatment (end of therapy for drug-sensitive TB). Peripheral blood mononuclear cells (PBMCs) were acquired from the patient after receiving their informed consent, as well as endorsement from the institutional review boards (No. N05/11/187; Health Research and Ethics Committee, Stellenbosch University). Frozen PBMCs were shipped to Sweden where they were HLA-typed using sequence-specific primer (SSP) typing kits (One Lambda Inc., Canoga Park, CA, USA). Ethical consent was also obtained from the ethics committee in Stockholm (Ref. 2011/863-31/2, including the samples from Zambia).

2.2. Cellular analysis with multimers

Thirty-eight fluorescently labelled multimers (streptavidin-phycoerythrin (PE), streptavidin-allophycocyanin (APC), and fluorescein isothiocyanate (FITC)) covering HLA-A*02:01, A*24:02, A*30:01, A*30:02, A*68:01, B*58:01, and C*07:01 presenting peptides from the MTB-derived proteins Rv0288, Rv1886c, Rv3875, Rv2958c, Rv2957, and Rv0447c, were either constructed in-house (as previously described²²) or commercially acquired (Beckman Coulter, San Diego, USA, and Immudex, Copenhagen, Denmark). In the CD3+CD8+CD4[−] compartment, multimer-positive events were recorded using anti-CD3-PE/Texas red (ECD) (Clone UCHT1; Beckman Coulter), anti-CD4-Pacific orange (Clone S3.5; Invitrogen, Carlsbad, CA, USA), and anti-CD8a-APC/Cy7 (Clone SK1; Becton Dickinson, Franklin Lakes, NJ, USA). Cells in the CD3+CD8[−]CD4⁺ compartment were excluded from

enumeration of multimer-positive events. All cellular analyses were performed using a FACS Gallios Flow-cytometer (Beckman Coulter). Only multimer responses that were at least three times higher than the negative control and for which we could detect more than 50 events were analyzed further.

2.3. Phenotypic analysis of the T-cells

Analysis of the phenotype, degranulation marker (CD107a), and survival marker (interleukin 7 receptor subunit alpha, IL7Rα) expression profile of the multimer-specific cells was performed using anti-CD45RA-PerCP/Cy5.5 (Clone HI100; Biolegend, San Diego, CA, USA), anti-CCR7-PE/Cy7 (Clone 3D12; Becton Dickinson), anti-CD107a-Pacific blue (PB) (Clone H4A3; Biolegend), and anti-CD127-APC/Alexa-700 (Clone R34.34; Beckman Coulter).

2.4. Statistical analysis

The Student's *t*-test or paired *t*-test was applied for the analysis of statistical significance between different T-cell populations using GraphPad Prism 4.0 software (GraphPad Inc., La Jolla, CA, USA). A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Multimeric analysis of selected TB epitopes

We successfully constructed 38 MHC class I MTB peptide-loaded multimers covering some of the most frequently occurring MHC class I alleles (HLA-A*02:01, A*24:02, A*30:01, A*30:02, A*68:01, B*58:01, and C*07:01) among South African and Zambian populations. The multimers were tested on samples obtained from seven HLA-typed individuals diagnosed with acute pulmonary TB, prior to and post anti-TB treatment. The antigen-specific anti-TB responses by CD8+ T-cells were generally low but diverse before and after treatment. Furthermore, the frequencies of antigen-specific CD8+ T-cells ranged between 0 and 2.2% before treatment, while the frequencies were found to range between 0 and 1.8% post anti-TB therapy (Table 1).

On average, the frequencies decreased significantly from 0.25% before treatment to 0.20% after treatment (*p* = 0.02) (Figure 1A), but the trend varied considerably between individual antigen-specific T-cell populations (Figure 1B). Anti-TB treatment resulted in only a limited decrease in frequencies of antigen-specific CD8+ T-cells for most multimer-specific populations. However, for a few multimer-specific populations, a dramatic decrease in frequencies could be observed, e.g., A2-TB10.4_{IMYNYPAML} (Δ 0.77%), A24-ESAT6_{ELNNALQNL} (Δ 0.54%), and B58-Ag85B_{QTYKWETFL} (Δ 0.34%). Despite an observed decrease in frequencies in the majority of antigen-specific CD8+ T-cell populations, an increase in antigen-specific T-cell frequencies could be observed post treatment with regard to some populations, e.g., T-cells directed against A2-Ag85B_{FIYAGSLSA}, A24-Ag85B_{IYAGSLSA}, and A68-TB10.4_{ANTMAMMAR} (Table 1). The average decrease in frequencies after anti-TB treatment was independent of allele-restricted and peptide-derived TB proteins. However, some T-cell populations restricted by certain HLA alleles exhibited the tendency for more dramatic decreases in T-cell frequencies post anti-TB treatment, e.g., B*58:01 (Δ 0.34%) and A*24:02 (Δ 0.12%). A decrease in frequency could also be observed with respect to antigen-specific T-cell populations recognizing peptides derived from the early expressed TB antigens (TB10.4, Ag85B, and ESAT-6) (Δ 0.11%) (Figure 1C) as compared to the glycosyltransferases and CFA synthase (Δ 0.01%) (Figure 1D). The decrease in frequencies for most multimer-specific populations was also true (Supplementary Material, Figure S1), despite the fact that some individuals (PAT1 and PAT4)

Table 1
Prevalence of epitope-specific T-cells identified by multimer staining^a

Tetramer/patient	1		2		3		4		5		6		7	
	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft	Bef	Aft
Neg PE	0.1	0.0	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.1	0.0	0.1
Neg APC			0.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.1	0.0	0.1
A2-TB10.4 _{IMYNYPAML}											0.8	0.1	1.0	0.2
A2-TB10.4 _{AMLGHAGDM}			0.2	0.1							0.1	0.2		
A2-TB10.4 _{MLGHAGDMA}			0.3	0.0							0.1	0.1		
A2-Ag85B _{YLLDGLRAQ}											0.0	0.1	0.3	0.5
A2-Ag85B _{KLIVANNTRL}											0.2	0.2	1.4	0.9
A2-Ag85B _{FIYAGSLA}											0.0	0.4	0.0	0.1
A2-ESAT6 _{AMASTEGNV}											0.1	0.1	0.4	0.4
A2-ESAT6 _{LLDEGKQSL}											0.0	0.1	0.0	0.1
A2-Rv2958 _{ALADLPVTV}											0.0	0.0	0.0	ND
A2-Rv2957 _{SIIPITLNV}											0.2	0.1	0.0	0.1
A2-Rv0447 _{VLAGSVDEL}											0.0	0.1	0.0	ND
A24-TB10.4 _{IMYNYPAML}					0.4	0.0	0.3	0.1						
A24-Ag85B _{WYQSGLSI}					0.2	0.2	0.5	0.3						
A24-Ag85B _{FLTSLPQW}					0.9	0.0	0.1	0.1						
A24-Ag85B _{IYAGSLAL}					0.0	0.4	0.0	0.0						
A24-ESAT6 _{AYQGVQKQW}					0.0	0.0	0.1	0.1						
A24-ESAT6 _{ELNNALQNL}					1.9	1.2	2.2	1.8						
A24-Rv2958 _{KYIAADRKI}					0.0	0.0	0.0	0.1						
A24-Rv2957 _{PYNLYRYVL}					0.3	0.1	0.1	0.4						
A24-Rv0447 _{KYIFPGGLL}					0.0	0.0	0.0	0.1						
A3001-TB10.4 _{QIMYNYPAM}			0.3	0.2					0.2	0.1				
A3001-TB10.4 _{LVRAYHAMS}			0.2	0.2					0.5	0.3				
A3001-Rv2957 _{IVLVRRWPK}			0.1	0.2					0.3	0.3				
A3002-TB10.4 _{QIMYNYPAM}			0.3	0.2					0.2	0.4				
A3002-TB10.4 _{IMYNYPAML}			0.7	0.2					0.2	0.2				
A3002-TB10.4 _{AMEDLVRAY}			0.2	0.2					0.3	0.1				
A3002-ESAT6 _{AMASTEGNV}									0.1	0.1				
A3002-Rv2958 _{SARLAGIPY}			0.5	0.2					0.8	0.6				
A3002-Rv0447 _{RMWELYLAY}			0.1	0.2					0.3	0.2				
A68-TB10.4 _{HAMSSTHEA}											0.3	0.2		
A68-TB10.4 _{ANTMAMMAR}											0.1	0.2		
A68-Ag85B _{LPQWLSANR}											0.2	0.1		
A68-Ag85B _{WGAQLNAMK}											0.2	0.1		
A68-Rv2958 _{AAPEPVARR}											0.2	0.1		
A68-Rv2957 _{LVYGDVIMR}											0.0	0.2		
A68-Rv0447 _{AASAAIANR}											0.0	0.0		
B58-Ag85B _{QTYKWTFL}	0.0	0.0					0.0	0.0			1.1	0.1		
Cw07-Ag85B _{ANNTLWVY}					0.0	0.0	0.1	0.0						

Bef, before therapy; Aft, after therapy; PE, streptavidin–phycoerythrin; APC, streptavidin–allophycocyanin; ND, not determined; PBMC, peripheral blood mononuclear cells; TB, tuberculosis; MHC, major histocompatibility complex.

^a PBMCs from individuals with TB were incubated with MHC-matched MHC class I TB multimers and stained for T-cell markers. Results are reported as the percentage multimer-positive events in the CD3+CD8+ T-cell population; negative gating was performed to exclude CD4+ T-cells. Negative multimers were included to decipher the background staining. For shaded points it was possible to retrieve the phenotype as well as data regarding the degranulation marker CCR7 and the survival marker CD127 of the antigen-specific T-cells.

remained culture-positive for MTB after 6 months of anti-TB treatment (information not shown).

3.2. Phenotypic analysis of antigen-specific T-cells

Phenotypic analyses of total CD8+ T-cells as well as the antigen-specific T-cell compartment based on CD45RA and CCR7 expression were performed to gauge differences before and after anti-TB treatment. In general, the majority of total as well as antigen-specific CD8+ T-cells belonged to the precursor compartment (CD45RA+CCR7+), followed by terminally differentiated (CD45RA+CCR7−) and effector memory cells (CD45RA−CCR7−). Importantly, the frequencies of these populations did not differ considerably prior to and post anti-TB treatment. The central memory T-cell compartment (CD45RA−CCR7+) decreased significantly post anti-TB treatment, both among total CD8+ T-cells ($p = 0.04$) and antigen-specific T-cell populations ($p = 0.008$) (Figure 2). The frequencies of antigen-specific CD8+ T-cells belonging to a certain phenotype (based on CD45RA/CCR7 expression) were independent of the presenting HLA allele and the antigen-derived MTB epitope (data not shown).

3.3. Analysis of degranulation and survival markers

IL7R α is an important marker for T-cell survival, and this receptor can normally be found on most mature T-cells.²³ No difference could be detected in terms of IL7R α (also known as CD127) expression among total CD8+ T-cells prior to (80%) and post treatment (78%). However, a significant decrease in IL7R α was detected among antigen-specific T-cells (88% vs. 83%; $p = 0.04$) (Figure 3A and B).

CD8+ T-cell cytotoxicity can be correlated with the expression of the degranulation marker CD107a (LAMP-1).²⁴ The expression of CD107a was generally upregulated on antigen-specific T-cells compared to the total CD8+ T-cell population. A trend of decreased frequencies of CD107a expression post-treatment could be seen among total CD8+ T-cells, while the opposite was true for the antigen-specific CD8+ T-cell populations (Figure 3C and D).

4. Discussion

The use of a broad panel of 38 different multimers presenting peptides from different MTB-derived proteins in this study allowed

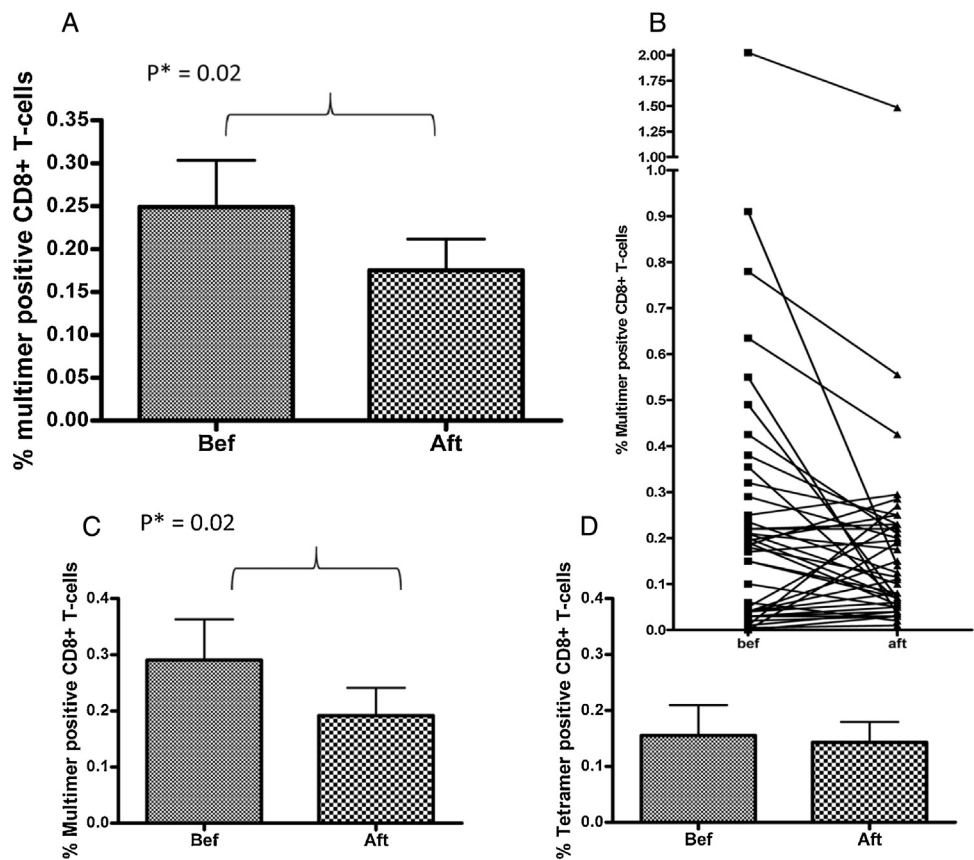


Figure 1. Frequency percentages of multimer-positive CD8+ T-cells. (A) Average detection of antigen-specific CD8+ T-cells in blood from patients before anti-TB treatment and after 6 months of completed treatment. (B) The trend of each of the 38 antigen-specific T-cell populations detected by different multimers before and after anti-TB treatment in the CD8+ T-cell compartment. The average percentage of multimer-specific CD8+ T-cells before and after anti-TB treatment recognizing epitopes derived from (C) the previously well-studied antigens TB10.4 (Rv0288), Ag85B (Rv1886c), and ESAT-6 (Rv3875), and from (D) the newly discovered TB antigens glycosyltransferase 1 (Rv2958c), glycosyltransferase 2 (Rv2957), and CFA synthase (Rv0447c). The two-sided Student's *t*-test was performed and significant values were calculated based on *p*-values; **p* < 0.05. (Bef, before therapy; Aft, after therapy.)

us to characterize the TB-specific CD8+ T-cell repertoire before and after (6 months) anti-TB treatment in patients from countries endemic for TB.²⁵ Since the importance of CD8+ T-cells has been increasingly acknowledged, e.g., in combination with anti-tumour necrosis factor alpha (anti-TNF- α) treatment,²⁶ it is important to further characterize the CD8+ T-cell-mediated immune response in patients with active TB, and identify the hitherto unknown effect of anti-TB treatment on antigen-specific T-cell populations thereof.

The effect of anti-TB treatment concerning the frequency, phenotype, and effector functions of antigen-specific CD8+ T-cells has been studied previously in different settings, involving adults and children with active TB.^{2,10,21,27,28} However, the respective outcomes of these studies have been conflicting. In the current report, the decreased frequencies of the antigen-specific CD8+ T-cells detected after TB treatment were contradictory to data reported by other groups evaluating TB-specific multimer staining

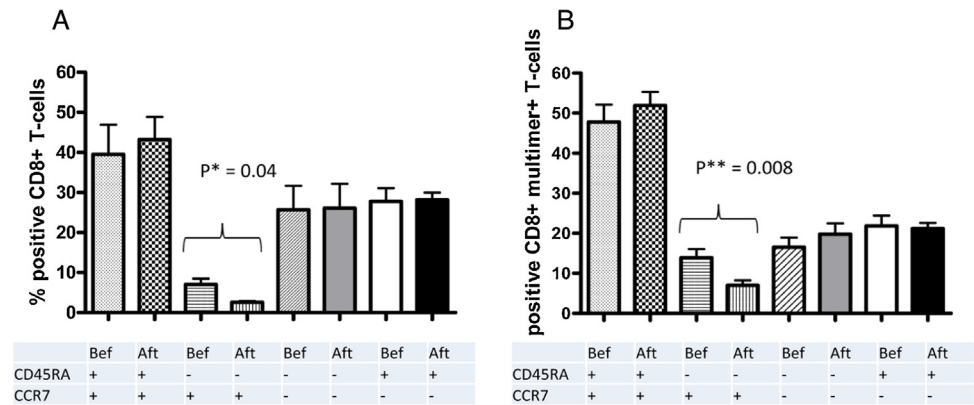


Figure 2. Frequencies of the (A) total CD8+ T-cells, and (B) antigen-specific CD8+ T-cells expressing different phenotypic markers before (naive (CD45RA+CCR7+), dotted; central memory (CD45RA-CCR7+), horizontal lines; effector memory (CD45RA-CCR7-), diagonal lines; terminally differentiated cells (CD45RA+CCR7-), white) and after anti-TB treatment (naive (CD45RA+CCR7+), chequered; central memory (CD45RA-CCR7+), vertical lines; effector memory (CD45RA-CCR7-), grey; terminally differentiated cells (CD45RA+CCR7-), black). The two-sided Student's *t*-test was performed and significant values were calculated based on *p*-values; **p* < 0.05. (Bef, before therapy; Aft, after therapy.)

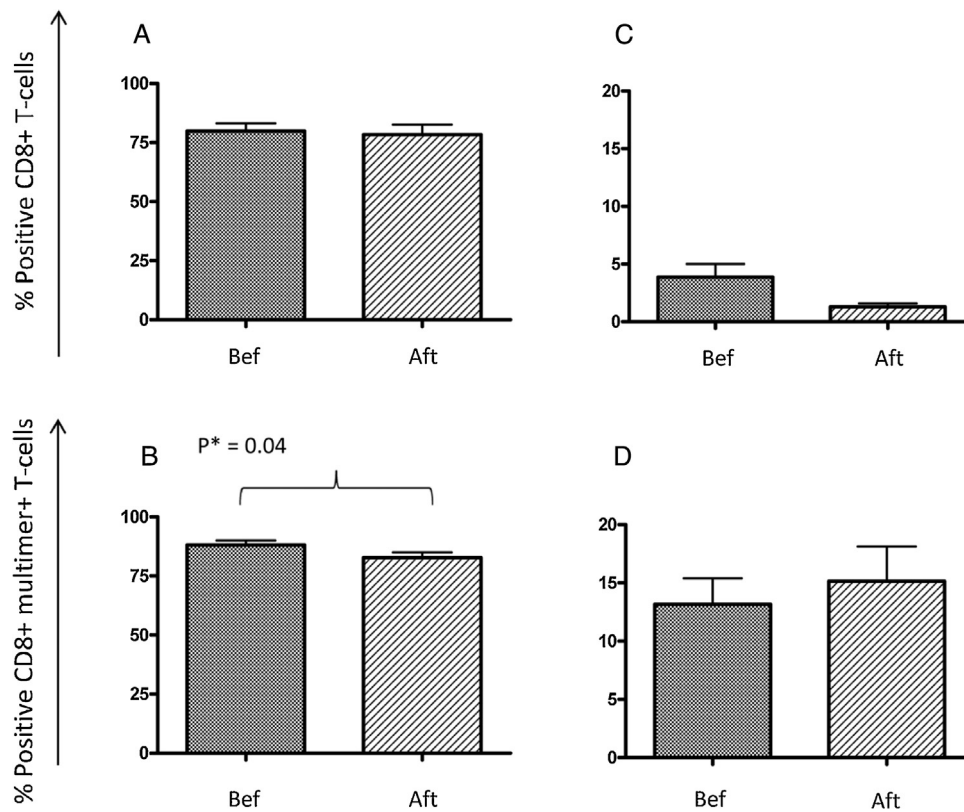


Figure 3. Frequencies of (A) total CD8+ T-cells, and (B) antigen-specific CD8+ T-cells expressing the CD127 (IL7R α) cell-surface marker before and after anti-TB therapy. Frequencies of (C) total CD8+ T-cells, and (D) antigen-specific CD8+ T-cells expressing the CD107a (LAMP-1) degranulation marker before and after anti-TB therapy. The two-sided Student's *t*-test was performed and significant values were calculated based on *p*-values; **p* < 0.05. (Bef, before therapy; Aft, after therapy.)

prior to and post anti-TB treatment. One reason for this might be the low patient number included in our study ($n = 7$), but also other points are worth discussing. One study described an increase in the frequency of Ag85A-specific CD8+ T-cells (0.4%) at 4 months post treatment in children using MHC class I multimers.²⁰ The same group also described a similar trend in antigen-specific T-cells (specific for the 16 kDa antigen, Rv1490 and ESAT-6) derived from MTB-infected adults. However, the results are not conclusive since the frequencies of antigen-specific CD8+ T-cells recognizing peptides derived from other TB antigens (Hsp65, Ag85B, and Rv1614) show similar or reduced frequencies of antigen-specific T-cells post anti-TB treatment.¹⁰ Also, a decrease in antigen-specific CD4+ T-cells could be seen post anti-TB treatment using HLA-DR8 multimers presenting ESAT-6-derived peptides.²⁹ Since our study shows a decrease in frequency of antigen-specific CD8+ T-cells particularly directed against the highly-expressed early antigens TB10.4, Ag85B, and ESAT-6, the decrease might be a result of reduced antigenic load during/after treatment (discussed below). However, this and other studies implementing MTB-specific MHC class I multimers, suggest that there appears to be a broad anti-TB CD8+ T-cell response involving many different epitopes derived from different groups of TB proteins, albeit at a very low frequency.^{10,19}

The phenotype of the total CD8+ T-cell and antigen-specific populations therein showed a similar pattern in our study, with a significant decrease in the central memory population post TB treatment. Furthermore, the size of the other phenotypic CD8+ T-cell compartments was not affected by anti-TB treatment. In line with our findings, a report published last year attested to the informative role of CD8+ T-cells in sensing bacterial burden in patients who had undergone standard anti-TB treatment. Since CD8+ T-cells recognize and respond to intrinsic (intracellular)

antigens, the authors claimed that a declining CD8+ T-cell response may be indicative of diminishing MTB populations due to anti-TB drug therapy.²¹ Nonetheless, our current report is the first study to shed light on CD8+ T-cells specific for several TB epitopes based on multimer staining.

Another important point is whether mycobacterial burden in the patient affects the maintenance of antigen-specific memory CD8+ T-cell numbers. In an evaluation by Theron and colleagues of the longitudinal T-cell reactivity of South African TB patients using interferon gamma release assays (IGRAs) such as TSPOT.TB, QuantiFERON Gold In-tube, and PPD-ELISpot in the backdrop of anti-TB treatment, mycobacterial load in sputum was found not to correlate with the amount of interferon gamma (IFN- γ) secreted by the patients' PBMCs.³⁰ Although IGRAs largely account for MTB-specific CD4+ T-cell responses in the periphery,³¹ CD8+ T-cells also contribute with IFN- γ secretion in response to stimulation with epitopes from the same antigens.³² Patients with TB who were culture-positive for MTB at the time of diagnosis and converted after 6 months of anti-TB treatment did not exhibit differences in IFN- γ levels. The authors concluded that cellular immune responses, at least in part, are not directly affected by MTB load in patients in high-burden countries for TB. A more recent study by Rozot et al. evaluated the cytokine profile of CD4+ and CD8+ T-cell response in patients with active TB as well as those with LTBI, originating from various high-burden countries for TB. Similar to the study by Theron and colleagues, MTB culture positivity was shown not to affect the antigen-specific T-cell response of the patients, while polyfunctionality was postulated as a reliable diagnostic measure of the disease state.²⁷ Nevertheless, TB serodiagnostic tests do not account for qualitative variations in TCR (T-cell receptor) specificities arising from different clusters of MTB antigens occurring in the patient following anti-TB treatment.

Several reasons may explain the different results concerning the number of antigen-specific T-cells in the course of TB therapy. The patient's genetic background, which reflects the predominantly expressed HLA alleles in a given population, determines which MTB epitopes the CD8+ T-cells are able to encounter and respond to. Other factors are the molecular modifications harboured by the MTB strain(s) infecting the individual, bioavailability of MTB-derived factors, and previous bacille Calmette–Guérin (BCG) vaccination, as well as contact with environmental mycobacteria that play a fundamental role in shaping the TCR antigen specificity repertoire.^{33,34} These phenomena impose continuous exposure of the infected individual to antigenic stress, collectively contributing to counter-modulation of T-cell functionality – and quality of responsiveness of individual T-cells. High rates of MTB transmission in high-burden countries for TB such as South Africa and Zambia is another inevitable factor that influences the diversity of CD8+ T-cell-dependent antigen recognition and the ensuing effector response.²⁵ It is important to appreciate that geographical variation and disease endemicity are critical parameters that affect anti-TB CD4+ and CD8+ T-cell responses observed in the population, a phenomenon that has been termed 'antigenic editing of the T-cell repertoire' in the cancer field.³⁵

High antigen concentration in an individual prior to initiation of therapy may induce the generation of potentially high numbers of antigen-specific T-cells, given that MTB bacillary load in a patient with pulmonary TB can reach 10^9 viable bacteria based on sputum-smear microscopy and culture.³⁶ As mentioned earlier, the majority of drug-sensitive MTB bacilli are killed in the process of anti-TB treatment, sparing only those that undergo metabolic and physiological adaptation and become refractory to antibiotics.³⁷ This serves as an indication of antigen turnover in the patient, shaping the repertoire while influencing shifts in bioavailability of MTB epitopes presented in association with their MHC class I/II background together with pro-inflammatory cytokines. Furthermore, there is a lack of knowledge concerning whether anti-TB treatment drives surviving MTB bacilli to a latent (dormant) state in humans, such as that observed in the Cornell mouse model of TB.^{37,38} Thus, the significant reduction in mycobacterial load due to either bacillary death or dormancy following anti-TB therapy is likely to alter the antigen recognition repertoire of available T-cells. The remarkable reduction in frequencies of IL7R α -expressing antigen-specific CD8+ T-cells after anti-TB therapy supports this notion. Therefore, some populations of antigen-specific T-cells that were initially primed by productive infection are possibly deprived of their cognate antigenic target(s) after anti-TB therapy, resulting in T-cell death in the long run.³⁹

A noteworthy point is the fitness and functionality of the T-cells themselves. The T-cell response to stimuli can be impaired in the event of excessive inflammation or anergy, due to the downregulation of CD3 zeta chain expression, which directly affects TCR activation.⁴⁰ A similar phenomenon has already been observed in pulmonary TB and leprosy where T-cell activity could be restored with the addition of exogenous interleukin 2 (IL-2).⁴¹ Since pulmonary TB is a disease characterized by a largely TNF- α -driven, tissue-destructive, hyper-inflammatory environment in the lung,¹⁶ the quality of antigen-specific T-cell responses is inevitably perturbed. This has very recently been reconfirmed in patients with active TB who present with a large proportion of TNF- α single-positive CD4+ T-cells in peripheral blood.²⁷ Besides downregulation of expression, delayed recruitment of the CD3 zeta chain to the TCR complex owing to binding of low-affinity antigen also contributes to reduced CD8+ T-cell functionality.⁴²

The fact that the central memory CD8+ T-cell frequencies in blood significantly declined following anti-TB therapy could

imply that these cells home to tissue or organ compartments. Okhrimenko and colleagues recently showed that the bone marrow harbours resting memory CD4+ and CD8+ T-cells alike, thus establishing a niche for long-term memory T-cells.⁴³ Homing of antigen-experienced memory CD8+ T-cells to the lung following anti-TB therapy is still under debate, considering that active human TB granulomas do not harbour functional CD8+ T-cell populations.^{44,45} Indeed, terminally differentiated CD45RA+CCR7– CD8+ T-cells have been associated with host-protective immune responses in LTBI.¹⁰ These findings suggest that the lack of functional CD8+ T-cells in active TB granulomas may, in part, be responsible for decreased immune surveillance. If anti-TB therapy prompts enhanced trafficking of MTB-specific memory CD8+ T-cells to tissue compartments, it may justify why their numbers in peripheral blood decreased with time.

In conclusion, our findings suggest that anti-TB therapy might alter the quality of CD8+ T-cell responses, based on the changes in antigen recognition. This is a crucial observation – anti-TB treatment may indirectly tailor the cellular immune compartment to better manage the disease. Our report provides a view of antigen-specific CD8+ T-cell populations that can in larger studies be used to evaluate and longitudinally monitor the effectiveness of anti-TB treatment and vaccine efficacy trials. This also applies to the assessment of novel host-directed therapies for TB, where the assessment of the quality of targeted and protective anti-MTB immune responses is desirable.

Acknowledgements

The study was funded in part by EDCTP TBNEAT (RAR, MB, MM, and AZ), and grants from VR, HLF, and Vinnova to MM. RAR received a grant from Karolinska Institutet (KID); AGI received an Innovation Postdoctoral fellowship from the DST/NRF.

Ethical approval: Peripheral blood mononuclear cells were acquired from the patient after receiving their informed consent, as well as endorsement from the institutional review boards (number N05/11/187; Health Research and Ethics Committee, Stellenbosch University). Ethical consent from the ethics committee in Stockholm was also obtained (Ref. 2011/863-31/2, including the samples from Zambia).

Conflict of interest: The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijid.2015.01.017>.

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